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Genetic changes, intra- and inter-specific introgression in farmed Nile tilapia (*Oreochromis niloticus*) in Thailand

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ABSTRACT

Fourteen microsatellite loci were used to examine genetic changes of four strains in Nile tilapia (*Oreochromis niloticus*) derived from genetically improved farmed tilapia (GIFT) and two strains derived from a local Chitralada strain of Nile tilapia in Thailand. Reference populations, including the ninth generation of GIFT strain, the original Chitralada strain, two conspecific reference populations from Ivory Coast and Uganda, and one population each of *Oreochromis mossambicus* and *Oreochromis aureus*, were also examined. Despite minor genetic changes, three of the four GIFT-derived populations retained their purity as GIFT while genetic variation did not decline. One of the GIFT-derived populations showed high levels of introgression from the Chitralada strain. Likewise, introgression from GIFT to the Chitralada-derived populations was seen. Inter-specific introgression from *O. mossambicus* was observed in the GIFT reference population and one of the Chitralada-derived strains. Introgression from *O. aureus* was detected in one of the GIFT-derived populations with a history of intensive inter-strain crossing. However, the introgression resulted in elevated genetic variation relative to the Chitralada original strains.

1. Introduction

World Nile tilapia (*Oreochromis niloticus*) production has been dramatically increasing recently (e.g. from 970,756 MT in 2000 to 2,334,432 MT in 2008; FAO, 2010). Before 1998, cultured stocks of Nile tilapia faced problems of genetic deterioration due to small numbers of founders and successive bottleneck effects (reviewed by Pullin and Capili, 1988; Eknath and Hulata, 2009). Introgression from the congeneric *Oreochromis mossambicus* (Macaranas et al., 1986; 1995) also presumably accounted for the decline in culture performance in some stocks (Amarasinghe and De Silva, 1996). Since 1997, the GIFT (Genetically Improved Farmed Tilapia) strain has been disseminated and has shown remarkable impacts in enhancing production of Nile tilapia (ADB, 2005; Ponzoni et al., 2010a). The GIFT and GIFT-derived strains have made significant contributions to major producer countries of Nile tilapia; e.g., they have accounted for 80% of the total tilapia seed production in China, 75% in Thailand, and 40% in the Philippines (Ponzoni et al., 2010a).

Thailand is among the world's top five producers of Nile tilapia (FAO, 2011), with annual production averaging 155,000 MT from 2000 to 2009 (FAO, 2011). Amongst freshwater commodities, Nile tilapia contributed more than 40% to total aquaculture production of the country (Department of Fisheries, 2010) and it is a major export commodity, contributing 12,956 MT, valued at more than US$ 22.3 million in 2010 (Customs Department, 2011). Before 1998, aquaculture of Nile tilapia in Thailand relied on the Chitralada strain, which originated from an Egyptian stock that reached Thailand in 1965 *via* Japan (Damrongratana and Kessanchai, 1966). Despite a lack of scientific evidence, the Chitralada-derived stocks were believed to suffer genetic deterioration and poor culture performance caused by poor broodstock management and introgression with *O. mossambicus* as was reported in other Asian strains of Nile tilapia (Gupta and Acosta, 2004; Macaranas et al., 1986). Therefore, when the GIFT strain was introduced to Thailand in 1998 (ADB, 2005), it was well accepted by major hatcheries across the country.

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It is of concern that genetic change may have occurred in the GIFT-derived strains in Thailand. Generally, genetic change in hatcheries is common and usually is accompanied by loss of alleles due to genetic drift, especially when effective population size (N_e) is small (Aho et al., 2006; Allendorf and Phelps, 1980; Coughlan et al., 1998; McKinna et al., 2010; Romana-Eguia et al., 2005). Subsequently, if N_e is continually low, inbreeding will accumulate (Falconer and Mackay, 1996; Romana-Eguia et al., 2005). Moreover, reduction of genetic variation may be triggered by selection (e.g. Appleyard and Ward, 2006), which has been practiced in some tilapia hatcheries (Prayad Soda, pers. comm.). Despite an inconclusive relationship between genetic variation based on molecular markers and performance (e.g. Borrell et al., 2004; Heath et al., 2002; Overturf et al., 2003; Shikano and Taniguchi, 2002), loss of genetic variation was assumed to cause the decline of Nile tilapia production in Fiji (McKinna et al., 2010). Introgression of genes from other species may occur and may also have adverse impacts on production (Amarasinghe and De Silva, 1996).

The critical questions of the present study are: 1) whether genetic change, especially loss of genetic diversity, has occurred in the GIFT derived strains in Thailand; and 2) did introgression occur from the local Chitralada strain and/or *O. mossambicus* into the GIFT derived strains and *vice versa*?

This study provided a scenario which is useful for broodstock management of other tilapia broodstocks in that: 1) broodstock management of the GIFT-derived strains in Thailand followed good broodstock management regimes, mainly by using large number of brooders and equal contribution by family; 2) intra-specific introgression resulted in increasing genetic variation of populations with low genetic variation; 3) genetic differentiation among the GIFT derived strains occurred after a few generations and may eventually result in valuable genetic resources for further genetic improvement; and 4) inter-specific introgression was frequently observed and the impact was still inconclusive.

2. Materials and methods

2.1. Fish samples: origins, collection and DNA extraction

Four GIFT-derived strains, i.e. strains that were intentionally founded with the GIFT strain and/or incorporated the GIFT strain as a resource strain, were collected from two private and two government hatcheries during February to September 2006; two Chitralada-derived strains were collected from a private and a government hatchery (Table 1). The sampling was limited to hatcheries that maintain their own broodstock and have been producing more than 10 million tilapia fingerlings per year for at least 10 years.

Six categories of reference populations were included (Table 1): a GIFT population, a Chitralada population, pure cultured and wild *O. niloticus* from Africa and a population each for *O. mossambicus* and *Oreochromis aureus*. *O. aureus* was included in this study because it was introduced to Thailand in 1980 for production of all-male tilapia (Tangtrongpiros, 1980) through interspecific hybridization with *O. niloticus*.

A piece of caudal fin (about 50 mg) was collected from each individual broodfish, stored in 95% ethyl alcohol and delivered to the Fish Genetics Laboratory, Department of Aquaculture, Faculty of Fisheries, Kasetsart University, Bangkok. DNA extraction was performed using the standard protocol of Taggart et al. (1992) with a slight modification: the DNA pellets were resuspended in TE buffer (10 mM Tris–HCl pH 7.5; 1 mM EDTA pH 8.0) and stored at −20 °C until use. The quantity and quality of the extracted DNA were determined by spectrophotometry and agarose gel electrophoresis.

2.2. Microsatellite primers and PCR conditions

Fourteen microsatellite primers developed from DNA of *O. niloticus* by Lee and Kocher (1996) were used (Table 2). A single-locus PCR for each of six microsatellite primers (*UNH172*, *UNH211*, *UNH216*, *UNH222*,

Table 1
Sources of six Nile tilapia hatchery populations in Thailand; four conspecific reference populations from Chitralada Villa Royal Residence Thailand (ON-CD), Uganda (ON-U), Ivory Coast (ON-I) and genetically improved GIFT strain (ON-GIFT); and one population each of *Oreochromis mossambicus* (from South Africa, OM-S) and *O. aureus* (from Egypt, OA-E); N = sample size.

Stock abbreviation	Names of hatcheries/locations	Origin of the stock	N
GIFT derived populations			
ON-PT	Pathum Thani Fisheries Test and Research Center (Pathum Thani FTRC)	Founders comprised fifty families of the 9th generation GIFT from the WorldFish Center, Malaysia in 2000, undergone within family selection for growth for 5 generations	50
ON-UT	Uttaradit Fisheries Test and Research Center (Uttaradit FTRC)	Founders comprised 33 out of 50 families of the 9th generation of GIFT from Pathum Thani FTRC, undergone 3 generations of within family selection for growth	50
ON-PB	Nam Sai Farm, Prachin Buri province	Founders comprised the 5th and 9th generation GIFT from the National Aquaculture Genetics Research Institute (NAGRI), Thailand in 1997 and 2000, respectively; no records on number of families, the stocks were merged and subjected to 2 generations of selection for growth	50
ON-CP	Charoen Pokphand Hatchery, Ayutthaya province.	Founders comprised 250 full-sib families using females from 2 sources, GIFT from Pathum Thani FTRC and GIFT originated in the Philippines, and males from Chitralada and other tilapia strain/species from Africa, undergone 5 generations of within family selection for growth	50
Chitralada derived populations			
ON-AIT	Asian Institute of Technology (AIT), Pathum Thani province	Founders comprised members of Chitralada strain reared in Chitralada Villa Royal Residence, Bangkok, no information on number of founders, undergone at least 10 generations of mass selection for growth	50
ON-AY	Rom Sai Farm, Ayutthaya province.	Founders comprised a combination of Chitralada strain and GIFT [GIFT was un-intentionally introduced due to the confused naming of the GIFT strain as Chitralada III (ADB, 2005; Chinnabut et al., 2007)], undergone 2 generations of mass selection for growth	50
Reference populations			
ON-CD	Chitralada population,	Collected from Chitralada Villa Royal Residence, Bangkok	80
ON-GIFT	GIFT population	The 9th generation GIFT originally collected from the Philippines (obtained from N. Taniguchi, Tohoku University, Japan)	28
ON-I	Ivory Coast population	A cultured population from Ivory Coast (obtained from K. Veverica, Auburn University, USA)	20
ON-U	Uganda population	Wild, collected from Lake Albert in Uganda (obtained from W. Mwanja, Uganda)	20
OM-S	Reference population for *O. mossambicus*	A cultured population from South Africa (obtained from G. Hulata, Agricultural Research Organization, Israel)	40
OA-E	Reference population for *O. aureus*	A cultured population from Egypt, (obtained from Mahmoud Rezk, WorldFish Center, Egypt)	40